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Chemotaxonomic study of three Artemisia species growing in Sinai, Egypt

by

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ABSTRACT

A comparative chemotaxonomic study of three *Artemisia* species, *A. monosperma*, *A. judaica* and *A. herba alba* is presented. Eight forms of *A. monosperma*, growing in Wadi El-Arish (North Sinai) and the other two species growing in Wadi El-Sheikh and Wadi El-Talaa (Saint Catherine, South Sinai), respectively, were collected in the same growth season to eliminate the effect of ecological factors.

The chemical study comprised the preliminary phytochemical screening, investigation of total, watersoluble and acid-insoluble ash; carbohydrates; total nitrogen and amino acids; lipids; fatty acids and flavonoids. The results revealed that the eight forms of *A. monosperma* were greatly similar in their chemical composition. On the other hand, they differed qualitatively and quantitatively from the other two species, viz. *A. herba alba* and *A. judaica*. Therefore, the phytochemical results fully justified the systematic treatment.

INTRODUCTION

The genus *Artemisia* is of common use in folk medicine and in pharmaceutical preparations (BOULOS, 1983), and several compounds were isolated from its tissues. Most of these compounds are of medicinal interest. In this regard FAHMY et al. (1960) isolated four crystalline compounds from powdered leaves and flowering tops of *A. monosperma*. MAKSUDOV et al. (1962) determined the essential oils, organic acids, tannins, sugars, ash and tars in blooms of *A. scoparia*.

Many authors identified and isolated a great number of flavone compounds from different *Artemisia* species: RODRIGUEZ et al. (1972) from seven *Artemisia* taxa; SEGAL et al. (1973) from *A. herba alba*; KHAFAGY et al. (1979) from *A. monosperma*; GHAZOULY et al. (1984) and BACHA (1984) from *A. judaica* and SALEH et al. (1985) from *A. monosperma* and *A. herba alba*, SAYED et al. (1979) studied the fatty acids of *A. absinthium*, while LAIVANT and PROSKURNIKOVA (1965) studied the amino acids of the proteins of *A. rhodantha*, qualitatively, quantitatively, and their seasonal fluctuations during the developmental phases. GARRONE et al. (1973) examined the levels of free amino acids in *A. vulgaris* and *A. verlotorum*. Also, KHAMDMOV and CHAMSRKOV (1976) studied qualitatively the amino acids in *A. diffusa*, *A. halophila* and *A. turanica*. HAMMOUDA et al. (1978) isolated an acetophenone derivative and coumarins from *A. monosperma*.

ALEKSEEVA (1962) studied the metabolism of carbohydrates in *A. turanica* in various soils under desert conditions. MAKI (1968) isolated hemicellulose from leaves of *A. capillaris*. On the other hand, GARRONE et al. (1973) examined the levels of soluble carbohydrates in *A. vulgaris* and *A. verlotorum*.

A critical taxonomical revision was realized by GAZARA (1987) for *Artemisia* species growing in Sinai and known earlier by TÄCKHOLM (1974). In this revision, it was possible to distinguish between different *Artemisia* species according to vegetative, head as well as floral characters. The following key was made by GAZARA (1987).

1.a Heads homogamous, oblong, tapering at base .....
..... *A. herba alba*

1.b Heads heterogenous, hemispherical to oblong-ovate, mot tapering at base 2

2.a Involucral bracts hairy, bisexual flowers fertile, 15-29 per head ..... *A. judaica*

2.b Involucral bracts glabrous, bisexual flowers sterile, 3-9 per head 3

3. Heads ovate, 4 mm long, female flowers 2-6 per head
..... *A. monosperma*

In the present study a chemotaxonomical investigation was carried out to compare the three *Artemisia* species, namely *A. monosperma*, *A. judaica* and *A. herba alba*, and between the eight different forms of the first species.

MATERIAL AND METHODS

The material used in the present investigation was obtained from *A. judaica, A. herba alba* and *A. monosperma* growing naturally in Sinai. The two first species were collected from South Sinai (Saint Catherine area). *A. judaica* was collected from Wadi El-Sheikh and *A. herba alba* from Wadi El-Talaa. The eigth different forms of *A. monosperma* (A1 - A8) were collected from Wadi El Arish, North Sinai. The plant samples of the three species were collected at the same grwoth season. The shoots were manually cleaned, dried in an oven at 50 C and reduced to fine powder.

The preliminary phytocehmical screening was carried out on the powdered dried shoots of the three different species of *Artemisia*. This included testing for volatile oils (BALBAA et al. 1981), tannins (A.O.A.C., 1975), unsaturated sterols (BRIESKORN et al. 1961), flavonoids (WALL et al. 1954 and BALBAA et al. 1981), glycosides and/or carbohydrates (VOGEL 1978), and saponins (WALL et al. 1954 and ABD EL MAKSOUD 1983).

The total ash, water-soluble ash as well as acid-insoluble ash were determined according to A.O.A.C. (1975) methodology, using two grams of the powdered air-dried shoots of the eight different of *A. monosperma* (A1 - A8), as well as *A. judaica* and *A. herba alba*.

The total carbohadrates were determined according to the A.O.A.C. (1975) method, and the sugar content was expressed as gram dextrose generally glucose per 100 gram dry weight. The qualitative investigation of the free and combined sugars was realized according to A.O.A.A. (1975) methods of analysis.

The total nitrogen content was determined by the microkjeldhal method (ALLEN et al. 1974). Amino acids and lipid contents were investigated according to ALLEN et al. (1974). Flavonoids were investigated according to A.O.A.C. (1975) and Bacha (1984) methodology. Finally, fatty acids were studied using gas - liquid chromatography according to A.O.A.C. (1975) methodology. The analysis was done by GCV chromatograph using the following conditions:

- Column	10 % PEGA
- Column temp.	70 C (initial temp.)
- Rate	8 C/min.
- Final temp.	190 C
- Final time	20 min.
- Chart speed	2 min./cm.
- N2	30 ml/min.
- H2	33 ml/min.
- Air	330 ml/min.

Table (1): Preliminary phytochemical screening of shoots of thress species of *Artemisia*

Test	Species		
	A. monosperma (A1-A8)	*A. judaica*	*A. herba alba*
Volatil oils	+ ve	+ ve	+ ve
Tannins	- ve	- ve	- ve
Unsaturated sterols	+ ve	+ ve	+ ve
Alkaloids	- ve	- ve	- ve
Flavonoids	+ ve	+ ve	+ ve
Gylcosides and/or	+ ve	+ ve	+ ve
Carbohydrates	+ ve	+ ve	+ ve
Saponins	+ ve	+ ve	+ ve

RESULTS AND DISCUSSION

Investigation of plant constituents:

The principal chemical constituents were studies in order to compare between the eight forms of *A. monosperma* on one hand and between the three *Artemisia* species on the other hand. It can be concluded from Table (1) that all the three species of *Artemisia* contained volatile oils, carbohydrates and/or glycosides, flavonoids, sterols and saponins. Negative results were obtained for tannins and alkaloids in all of them.

Results presented in Table (2), revealed that the percentages of total ash content were approximately similar in different forms of *A. monosperma* ranging between 7.5 and 8.0 g.%. On the other hand the percentages were 7.5. and 5.5 g.% in *A. judaica* and *A. herba alba* respectively. It is clear also that water-soluble ash content in *A. monosperma* were approximately similar in different forms ranging between 3.5 and 4.0 g.%. Obviously, the records were 4.0 and 2.5 g.% in *A. judaica* and *A. herba alba* respectively. Results also clarified that the acid-insoluble content of different forms of *A. monosperma* ranged between 0.5 and 1.0 g.%. *A. judaica* and *A. herba alba* on the other hand had higher values of 2.8 and 2.3 g.% respectively (Table 2).

It is clear that the eight forms of *A. monosperma* had similar contents of total ash as well as water-soluble and acid-insoluble ash content. These contents differed from those ot the other two *Artemisia* species. Although the total ash and water-soluble ash of *A. judaica* were similar to those of *A. monospermam* the acid-insoluble ash was much higher. The contents of the three types of ash in *A. herba alba* differed from those of the other two species.

Results presented in Table (2) clearly show that the total carbohydrate contents of the studied *Artemisia* species belonging to *A. monosperma* (A1 - A8) attained values that ranged between 0.83 and 0.879/100 g. dry matter. On the other hand, data collected for *A. judaica* and *A. herba alba* indicated higher values (2.42 & 2.60) g.% respectively).

The qualitative study of sugars presents in the three studied species using paper chromatography (Table 3) revealed that *A. monosperma* with all different forms contained galactose. The other two species, namely *A. judaica* and *A. herba alba* contained mannose. All the forms of *A. monosperma* differed from the other two *Artemisia* species.

Table (2) A: Quantitative Analysis of the three *Artemisia* species

Species / Characters	A. monosperma								A.judaica	A.herba alba
	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8		
Total Ash (g.%)	7.5	7.5	7.5	8.0	7.5	8.0	8.0	7.5	7.5	5.5
Water Soluble Ash (g.%)	4.0	4.0	3.5	4.0	4.0	4.0	4.0	4.0	4.0	2.5
Acid - Insoluble Ash (g.%)	0.5	1.0	1.0	0.5	0.5	1.0	1.0	0.5	2.8	2.3
Total Carbohydrates as g.% glucose	0.87	0.83	0.83	0.83	0.87	0.87	0.87	0.83	2.42	2.60
Total Nitrogen content g./100g. plant material	0.286	0.276	0.286	0.286	0.286	0.276	0.276	0.276	0.332	0.350
Percentage of alcoholic extract (crude flavones)	3.28	3.31	3.37	3.24	3.28	3.28	3.37	3.28	0.50	0.40
Total Lipid content g.%	15.4	15.4	14.2	15.4	14.2	15.4	14.2	14.2	10.2	8.2

Results in Table (2) also show that the amounts of the total nitrogen content attained their maximum values in *A. judaica* (0.332 g%) and *A. herba alba* (0.350 g.%) that decreased remarkably in the eight forms of *A. monosperma* (0.276-0.286 g.%).

The results of total lipid content (Table 2) estimated quantitatively in the different forms of *A. monosperma* (A1 - A8) indicated values that ranged between 14.2 to 15.4 g.%. On the other hand, both *A. judaica* and *A. herba alba* indicated lower values of 10.2 and 8.2 g.% respectively.

The percentages of the alcoholic extract, containing total flavones, were also compared (Table 2). It is evident that the crude total flavonoid content of the eight forms of *A. monosperma* were similar, ranging from 3.24 to 3.31 g.%; however *A. judaica* contained only 0.5 g.%; nevertheless *A. herba alba* contained the least amount of flavonoids (0.14 g.%).

The results of the qualitative study of amino acids in the three studied species using paper chromatography (Table 3) revealed that *A. monosperma* with all its different forms contained lysine, asparagin, aspartic acid, glutamic acid, alanine, tyrosine, methionine and leucine. On the other hand, *A. judaica* contained lysine, aspartic acid, glutamic acid, alanine, tyrosine, valine, and leucine; while *A. herba alba* contained cystine, lysine, asparagin, serine, alanine, valine and isoleucine.

Considering the qualitative investigation of flavonoids (Table 3) *A. monosperma* contained quercetin 3-glucoside, quercetin 3-rutinoside, quercetin 5-glucoside, insorhamnetin 5-glucoside, patuletin 3-rutinoside, acacetin 7-glucoside, acacetin 7-rutinoside, vicenin -2, lucenin and methylated aglycones as reported by SALEH et al. (1985). Differently, *A. judaica* contained chrysoerial 7-rutinoside, leutulin, cirstakogenin (BACHA 1984). Finally, *A. herba alba* contained quercetin 3-glucoside, quercetin 3-rutinoside, patuletin 3-glucoside, patuletin 3-rutinoside, isovitexin, vicenin -2 schaftoside, isoschaftoside and methylated aglycones (SALEH et al. 1985). These results clarify the presence of different flavones in the three *Artemisia* species. The results also show that the eight forms of *A. monosperma* contain the same flavones, and differ from the other two species.

The resulty presented in Table (4) reveal the presence of the following fatty acids in the eight forms of *A. monosperma*: octanoic, capric, lauric, tridecanoid, myristic, pentadecanoic, palmitic, stearic, oleic, linoleic, linolenic and arachidic acids. It is also clear that *A. judaica* contained a group of fatty acids similar to that of *A. monosperma*, with the difference that undecanoic acid is present, while octanoic and arachidic acids are absent. On the

Table (3): Qualitative analysis of Carbohydrates, amino-acids, flaronoids and fatty acids of the three *Artemisia* species

Species / Characters	*A. monosperma*								*A.judaica*	*A.herba-alba*
	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8		
Carbohydrates										
Galactose	+	+	+	+	+	+	+	+	-	-
Mannose	-	-	-	-	-	-	-	-	+	+
Amino acids										
Cystine	-	-	-	-	-	-	-	-	+	+
Lysine	+	+	+	+	+	+	+	+	+	+
Aspargine	+	+	+	+	+	+	+	+	-	+
Aspartic acid	+	+	+	+	+	+	+	+	+	-
Glutamic acid	+	+	+	+	+	+	+	+	+	-
Serine	-	-	-	-	-	-	-	-	-	+
Alanine	+	+	+	+	+	+	+	+	+	+
Tyrosine	+	+	+	+	+	+	+	+	+	-
Methionine	+	+	+	+	+	+	+	+	-	-
Valine	-	-	-	-	-	-	-	-	+	+
Leucine	+	+	+	+	+	+	+	+	+	-
Isoleucine	-	-	-	-	-	-	-	-	-	+
*Flavonoids										
Quercetin 3-glucoside	+	+	+	+	+	+	+	+	-	+
Quercetin 3-rutinoside	+	+	+	+	+	+	+	+	-	+
Quercetin 5-glucoside	+	+	+	+	+	+	+	+	-	+
Isorhamnetin 5-glucoside	+	+	+	+	+	+	+	+	-	-
Patuletin 3-glucoside	+	+	+	+	+	+	+	+	-	+
Paluletin 3-rutinoside	+	+	+	+	+	+	+	+	-	+
Acacetin 7-glucoside	-	-	-	-	-	-	-	-	-	+
Acacetin 7-rutinoside	+	+	+	+	+	+	+	+	-	-
Isovitexin	+	+	+	+	+	+	+	+	-	-
Vicenin - 2	+	+	+	+	+	+	+	+	-	+
Schaftoside	-	-	-	-	-	-	-	-	-	+
Isoschaftoside	-	-	-	-	-	-	-	-	-	+
Lucenin - 2	+	+	+	+	+	+	+	+	-	-
Methyaglycone	+	+	+	+	+	+	+	+	-	+
Chrysoerial 7-rutinoside	-	-	-	-	-	-	-	-	+	-
Leutulin	-	-	-	-	-	-	-	-	+	-
Cirstakogenin	-	-	-	-	-	-	-	-	+	-
Apigenin	-	-	-	-	-	-	-	-	+	-

* Identified by BACHA (1984) & SALEH et al. (1985).

Table (3) Qualitative analysis (Cont.)

Species / Characters	*A. monosperma*								*A.judaica*	*A.herba-alba*
	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8		
Fatty acid esters										
Octanoic	+	+	+	+	+	+	+	+	+	-
Capric	+	+	+	+	+	+	+	+	+	+
Undecanoic	-	-	-	-	-	-	-	-	-	-
Lauric	+	+	+	+	+	+	+	+	+	+
Tridecanoic	+	+	+	+	+	+	+	+	+	+
Myristic	+	+	+	+	+	+	+	+	+	+
Pentadecanoic	+	+	+	+	+	+	+	+	+	+
Palmitic	+	+	+	+	+	+	+	+	+	+
Stearic	+	+	+	+	+	+	+	+	+	+
Oleic	+	+	+	+	+	+	+	+	+	+
Linoleic	+	+	+	+	+	+	+	+	+	+
Linolemic	+	+	+	+	+	+	+	+	-	+
Arachidic	+	+	+	+	+	+	+	+	-	-

Table (4): Percentages of fatty acid esters of the three studied *Artemisia* species

Species / Fatty acid	*A. monosperma*								*A. judaica*	*A. herba-alba*
	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8		
Octanoic	5.84	0.82	9.09	22.57	5.75	6.85	4.59	0.82		25.87
Capric	26.16	9.33	12.12	4.27	16.09	21.27	25.22	9.33	24.31	7.71
Undecanoic	-	-	-	-	-	-	-	-	2.78	-
Lauric	1.11	0.27	0.22	1.65	0.81	2.95	0.33	0.27	8.10	8.81
Tridecanoic	0.47	1.51	2.98	5.37	2.01	5.32	3.49	1.51	2.31	12.11
Myristic	3.96	0.96	1.5	2.20	2.76	11.82	2.18	0.96	3.86	5.50
Pentadecanoic	4.42	16.58	9.20	34.17	9.58	14.65	4.80	16.58	27.39	4.41
Palmitic	50.49	15.79	17.86	11.3	21.86	24.82	16.38	15.79	1.93	2.75
Stearic	0.32	1.57	2.71	1.65	1.53	0.83	0.76	1.57	3.70	1.47
Oleic	0.26	9.02	8.44	3.05	7.66	0.59	15.07	9.02	4.63	17.61
Linoleic	3.47	36.84	18.99	8.79	25.21	0.89	16.81	36.84	7.41	13.76
Linolemic	2.24	5.26	12.99	3.67	3.58	2.92	8.73	5.26	13.58	-
Arachidic	1.26	2.05	3.90	1.22	3.16	7.09	1.64	2.05	-	-

other hand *A. herba alba* contained octanoic, capric, lauric, tridecanoic, stearic, oleic, linoleic, acids, and was free of undecanoic, lineolenic and arachidic acids. These results show that the percentage of some fatty acids varies not only in the three *Artemisia* species, but also in the forms of *A. monosperma* (Table 4). It must be noted that the qualitative estimation of fatty acids esters by Gas Liquid Chromotography is strict since it depends on comparing the fatty acids by authentic samples. On the other hand, the quantitative estimation of some fatty acids by the same method may differ sinde the peak shape differs in operating conditions and injection technique.

It can be concluded that the eight forms of *A. monosperma* contain the same fatty acids, which differ from those of the other two species viz. *A. judaica* and *A. herba alba*.

From these results, it is clear that the eight forms of *A. monosperma* are greatly similar in their chemical composition. They differ qualitatively and quantitatively from the other two species, viz. *A. judaica* and *A. herba alba*. In this regard, the phytochemical study fully justifies the systematic treatment.

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